

AD _____

Award Number: DAMD17-03-1-0261

TITLE: Serum Genetic Markers as Surrogates of Prostate Cancer Progression

PRINCIPAL INVESTIGATOR: Dave S.B. Hoon, Ph.D.

CONTRACTING ORGANIZATION: John Wayne Cancer Institute
Santa Monica, CA 90404

REPORT DATE: April 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 01-04-2007		2. REPORT TYPE Annual		3. DATES COVERED 1 Apr 2006 – 31 Mar 2007	
4. TITLE AND SUBTITLE Serum Genetic Markers as Surrogates of Prostate Cancer Progression				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-03-1-0261	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dave S.B. Hoon, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) John Wayne Cancer Institute Santa Monica, CA 90404				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT The main purpose of the proposal is that detection of free tumor-related DNA marker(s) in serum can be used as surrogate genetic markers for monitoring ongoing events related to the pathogenesis of metastasis and provide prognostic insight into disease outcome and treatment response. The scope of the studies is to develop and validate tumor-related circulating DNA in serum of prostate cancer (PCA) patients. The goal is to validate these DNA markers. We have developed assays for circulating serum DNA in prostate cancer patients (PCA). The program has gotten on track in the past year since the approval of the Human subjects IRB. PCA patient's and normal donor serum was accrued in the past year. We have been screening for new circulating methylated and unmethylated tumor-related DNA markers. Several DNA markers were found to correlate with PCA stage significantly. Highly sensitive assays were developed to detect these DNA markers in serum. Optimal conditions to obtain high specificity and sensitivity were determined. In the coming year more PCA patients will be accrued as well as further accrual of normal age-matched donors for the study. Assays for the markers will be carried out. We plan to complete the study in the upcoming year.					
15. SUBJECT TERMS Circulating DNA, serum, prostate cancer, methylation, PCR					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

Body.....	4
Key Research Accomplishments.....	8
Reportable Outcomes.....	8
Conclusions.....	8
References.....	8
Appendices.....	8

BODY

During this grant period, we have been accruing PCA patients and normal volunteer male donors. Last year, we received approval by the human subjects IRB at our institute and at Fred Hutchison Cancer Center (our collaborator), and progress in the past was due to patient accrual, an integral part of the proposal. We have made major progress and generated significant findings as discussed below. In the past year, we have accrued blood from PCA patients and healthy male donors from JWCI, Fred Hutchinson Cancer Center, and The Angeles Clinic, as described in the Statement of Work Task 1. The patients have been accrued from these three centers as outlined in the proposal. In this last period, we have been receiving patients from the Fred Hutchinson collaboration group.

Blood was processed as indicated in Task 1. The blood was purified and cryopreserved for later extraction or processed for DNA immediately, as outlined in the proposal. The serum DNA was isolated, purified, quantified, and aliquoted, as indicated in the tasks outlined for the proposal. These procedures were optimized to obtain maximum efficiency in isolating small amounts of DNA from the serum. Blood was processed to serum as indicated for Task 1. We have investigated various approaches in optimizing circulating DNA extraction from serum, and have an excellent procedure established. The focus has been to use less than 200 ul serum, representative of the patients' circulating DNA. In addition, we have been exploring the need to filter the serum for contaminating cells that may interfere in the assay. For methylation marker studies, filtration does not appear to be necessary. We have done side-by-side studies of filtered and non-filtered serum from the same patients and normal donors. Methylated and unmethylated DNA was assessed using specific markers by capillary array electrophoresis (CAE). For loss of heterozygosity of microsatellites (LOH) analysis, we also use the CAE instrument and software. In addition, we have developed realtime PCR analysis of methylated markers for validation of the results. This has been carried out with methylation-specific PCR primers labeled with specific TaqMan fluorescent dyes.

In the past year, we have been working on Task 2 in developing and establishing serum methylated DNA markers. We have screened multiple markers that include tumor-related genes. In 85 evaluable patients, we have assessed approximately 25 AJCC Stages I, II, III patients, and 58 AJCC stage IV patients. Other patients we have accrued still need to be assessed. For the methylation markers, we assessed RASSF1A, RAR β 2, and GSTP-1. All these methylation markers are tumor-related genes and are known to be methylated in prostate tumor tissue and not in normal prostate tissue. For the LOH studies, we assessed the microsatellite markers D6S286, D8S262, D8S261, D9S171, D10S591, and D18S70. LOH of these markers is not found in normal tissue.

DNA markers (methylated vs unmethylated) were compared to PSA quantity. Methylated RASSF1A and R status in 81 patients was significantly ($P=0.02$) correlated to PSA levels. Methylated RAR β 2 status in 81 patients was significantly ($P=0.017$) correlated to PSA levels. Methylated GSTP1 status in 81 patients was significantly ($P<0.001$) correlated to PSA levels. These analyses were performed independent of stage. The findings indicate that GSTP1, RAR β 2, and RASSF1A, are significant, respectively, correlated to PSA levels. See Figures 1 and 2 in methylated status of individual markers and all markers to PSA level in serum (ng/ml).

Figure 1: Marker Methylation vs. PSA Level

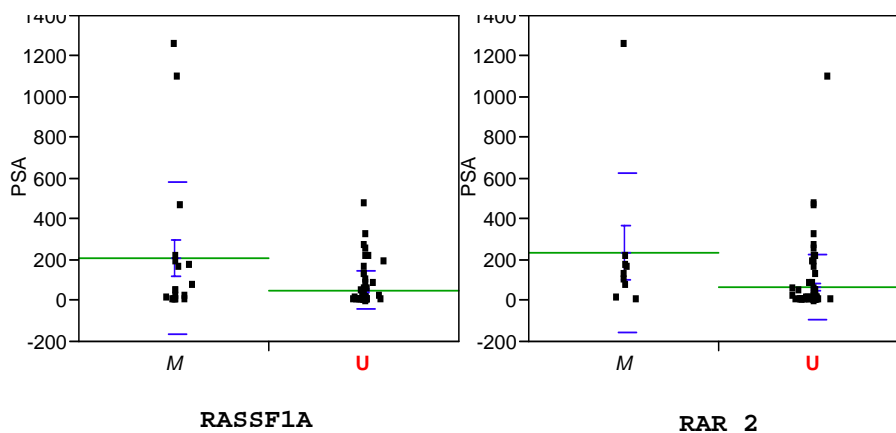
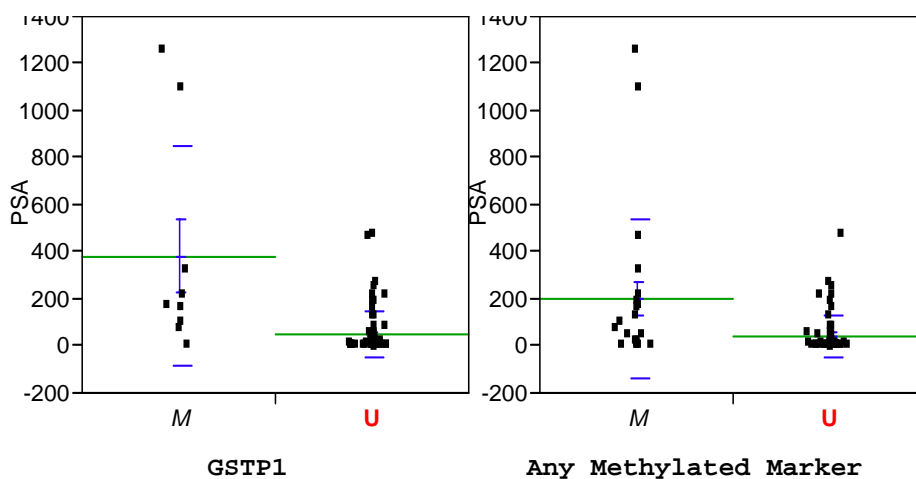


Figure 2: Marker Methylation vs. PSA Level



In Tables 1A,B,C, we show the detection frequency of markers, individual and in combination, relative to all stages, early stage, and advanced stage, respectively. The strategy of using two different types of markers for detection is to improve overall sensitivity. The frequency of LOH markers is higher in early stage disease compared to advanced stage. The presence of LOH markers was observed very frequently in early stage disease, significantly more so than methylated markers. Further patient accrual is needed, particularly for assessment of early stage disease.

Table 1A: PCA Detection by DNA Serum Markers (n=83)

Marker	%	Ratio
PSA 4.0	70	57/81
PSA 10.0	51	41/81
RASSF1A	24	20/83
RAR β 2	12	10/83
GSTP1	13	11/83
any methylation	29	24/83
any LOH	47	38/81
Methylation and/or LOH	61	51/83

**Table 1B: PCA Detection by DNA Serum Markers
Stage I, II, III (n=25)**

Marker	%	Ratio
PSA 4.0	40	10/25
PSA 10.0	4	1/25
RASSF1A	16	4/25
RAR β 2	0	0/25
GSTP1	0	0/25
any methylation	16	4/25
any LOH	60	15/25
Methylation and/or LOH	68	17/25

**Table 1C: PCA Detections by DNA Serum Markers
Stage IV (n=58)**

Marker	%	Ratio
PSA 4.0	81	47/58
PSA 10.0	69	40/58
RASSF1A	28	16/58
RAR β 2	17	10/58
GSTP1	19	11/58
any methylation	34	20/58
any LOH	41	23/56
Methylation and/or LOH	59	34/58

In Table 2, methylation of markers is compared to early and advanced stage disease. There was a significant correlation of both RAR β 2 and GSTP1 to more advanced disease stage. This demonstrated a difference in marker methylation status based on disease stage. The presence of either marker was more significant than an individual marker.

Table 2: Methylated DNA Markers Correlation to AJCC Stage

	Stage I, II, III	Stage IV	P Value
RASSF1A	4/25	16/5	0.401
RAR β 2	0/25	10/5	0.028
GSTP1	0/25	11/5	0.029
any methylation	4/25	20/5	0.116
RAR β 2 and/or GSTP1	0/25	13/5	0.008

In Table 3, methylation markers, individually and in combination, are assessed relative to Gleason Score (GS) in patients. GS is an important indicator of the pathology status of the primary tumor. Some clinicians consider this a more important prognostic factor than other factors, including stage of disease. We demonstrate that methylation status of both GSTP1 and RASSF1A were significantly correlated to GS, and that the combination of all 3 methylation markers approached significance. An increase in sample size will determine if the latter is significant. These are important and novel findings and will be further expanded. New methylation markers are being assessed that may improve the sensitivity of the assay to pathology status of the tumor. In Table 4, we report the frequency of LOH in serum of different stages of PCA. However, the results to date demonstrate no significant difference with advancing disease stage. The sample size needs to be increased to substantiate this finding.

Table 3: Correlation of Methylation of DNA Marker to Gleason Score

	GS LOW	GS HIGH	P Value
RASSF1A	6/39	11/30	0.05
RAR β 2	3/39	6/30	NS
GSTP1	2/39	7/30	0.0308
any methylation	8/39	12/30	0.067

Table 4: Correlation of LOH vs. AJCC Stage

	LOH (-)	LOH (+)
Stage I, II, III	10/25	15/25
Stage IV	33/56	23/56
P=0.00		

For Task 4, we are continuing accrual of patients receiving radiation therapy. In the upcoming year, we will continue collecting specimens of PCA patients being treated, as well as

early-stage patients. More early-stage patients are needed to balance the sample size distribution for evaluation of markers. The accrual of patients receiving radiation therapy has been slow due to the lengthy process of obtaining patient agreement in participation. We will complete this portion of the study and run the individual marker analysis. We will also assess new methylation markers in which we have identified and developed primers.

In the upcoming year, we will continue accruing patients from the different clinics outlined in the proposal. Data will be analyzed and a final report will be generated.

Key Research Accomplishments

1. PCA patients' accrual for blood.
2. Normal healthy male donors' sera accrual for blood.
3. Blood is processed for serum and cryopreserved.
4. Blood is processed for leukocytes as normal genomic controls.
5. Serum DNA is extracted, purified, and quantified.
6. DNA is cryopreserved.
7. DNA is processed from leukocytes.
8. Serum DNA assessed for LOH markers.
9. Serum DNA assessed for methylation markers.
10. Data from serum DNA markers correlated with patients' clinical status and prognostic factors.
11. Identification of significant correlation of serum DNA markers with patients' Gleason Score and stage of disease.

Reportable Outcomes

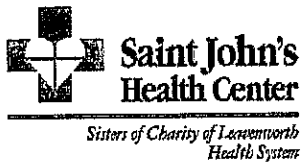
No reportable outcomes have occurred. Currently, we are in preparation for submitting one manuscript and writing up a second manuscript.

Conclusions

In the past year, we have been accruing more patients to reach our objectives from two clinical sites. The studies are progressing forward in terms of patient accrual. Serum from patients has been run on two different types of DNA biomarkers (LOH and methylation). The studies demonstrate that both types of biomarkers can be detected in PCA patients and not in normal donors. The biostatistical analysis of the current PCA patients indicates there is a significant correlation of the biomarkers with disease status and prognostic factors. The success of the studies may be of significant clinical utility in assessment of PCA patients.

References: None.

Appendices: Current IRB-approved Letter



Saint John's Health Center/John Wayne Cancer Institute
Joint Institutional Review Board (IRB)

February 14, 2007

Dave Hoon, Ph.D.
John Wayne Cancer Institute
2200 Santa Monica Blvd
Santa Monica, CA 90404

IRB Protocol Number: HOOD-PCR-0101

Protocol Title: Serum Genetic Markers as Surrogates of Prostate Cancer Progression: Detection of Circulating Markers in Patients Using Polymerase Chain Reaction (PCR)".

Principal Investigator: Dave Hoon, Ph.D.

Approved Co-Investigators: Robert Wollman, MD, Frederick R, Singer, MD, Tia Higano, MD, Tanya Dorff, M.D.

Protocol Version Date: October 18, 2006
Consent (Cancer Volunteer) October 18, 2006
Consent (Cancer Free Volunteer) October 18, 2006

Re: Clarification Regarding Correct Title of Study

Dear Dr. Hoon,

The Saint John's Health Center / John Wayne Cancer Institute Joint Institutional Review Board (IRB) inadvertently referenced the incorrect title of the above referenced study in IRB correspondence. The IRB did receive and reviewed/approved the change which was submitted with Amendment #2 and in addition to Amendment #5. The IRB has appropriately updated our electronic records and confirms that the correct title of the study is the following: "Serum Genetic Markers as Surrogates of Prostate Cancer Progression: Detection of Circulating Markers in Patients Using Polymerase Chain Reaction (PCR)." If you have any questions, please contact the IRB office.

Sincerely,


Kristin J. Allen, MPH, CIP
SJHC/JWCI Joint IRB Administrator/Member

Saint John's Health Center/John Wayne Cancer Institute
Joint Institutional Review Board (IRB)

October 31, 2006

David Hoon, Ph.D.
John Wayne Cancer Institute
2200 Santa Monica Blvd
Santa Monica, CA 90404

IRB Protocol Number: HOOD-PCR-0101

Protocol Title: Serum Genetic Markers as Surrogates of Prostate Cancer: Detection of Circulating Surrogate Markers of disease in Patients Undergoing Radiation Therapy Using Polymerase Chain Reaction (PRC) and Proteomics

Principal Investigator: David Hoon, Ph.D.

Approved Co-Investigators: Robert Wollman, MD, Frederick R, Singer, MD, Tai Higano, MD, Tanya Dorff, M.D.

Protocol Version Date: October 18, 2006
Consent (Cancer Volunteer) October 18, 2006
Consent (Cancer Free Volunteer) October 18, 2006

Re: Final Approval of Renewal # 6

Dear Dr. Hoon,

The Saint John's Health Center / John Wayne Cancer Institute Joint Institutional Review Board (IRB) granted conditional approval of Renewal # 6 of the above referenced protocol at the October 4, 2006 meeting pending changes to the informed consents.

The IRB noted that the requested changes have been made in addition to minor changes to the protocol and consents. Final approval of this renewal was granted on October 31, 2006.

Any proposed change in the protocol, or consent form must be submitted to the IRB and approved prior to implementation of the change. Any death of a patient on protocol regardless of cause must be reported in writing to the IRB within 72 hours after discovery. All serious and/or unexpected, as defined on the IRB reporting form, adverse events must be reported to the IRB in writing within 7 calendar days after discovery.

This study is approved for one additional year. **Approval expires October 3, 2007.**

If you wish to continue this protocol beyond one year, you must submit the required

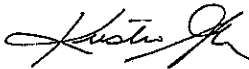
IRB Submission Materials for Continuing Review to the IRB Office **no later than August 20, 2007**, for review in the September 5, 2007 meeting.

You are required to notify the IRB using the required forms when:

- 1) the study stops accruing new subjects but protocol interactions continue with previously enrolled subjects,
- 2) protocol treatment of previously enrolled subjects ends,
- 3) the study is completed,
- 4) the study is cancelled.

These forms are now available on-line at the IRB website www.stjohns.org/irb

Sincerely,



Kristin J. Allen, MPH, CIP
SJHC/JWCI Joint IRB Administrator/Member